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INFORMATION ESSENTIAL FOR CHARACTERIZING A FLOW-BASED ANALYTICAL SYSTEM

(IUPAC Technical Report)

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Abstract: Essential aspects for characterization of a flow-based analytical procedure or system are discussed in order to permit the composition of a checklist that will lead to a protocol for reporting results and systems in flow analysis. Aspects more related to chromatographic procedures are not considered. The intent is to present normalized proposals in the field of flow analysis for practitioners and developers.

1. INTRODUCTION

Terminology related to classification and definition of analytical methods based on flowing media, as well as terms describing the flow-based analytical procedure or system and its components have already been presented [1–3]. However, a literature survey reveals that a number of such analytical procedures and/or related instrumentation are only partially described. As a proper description of any methodology is essential, it is important to complement the earlier recommendations by taking into account the recent progress in flow analysis.

The main objective herein is to provide guidelines for characterizing a flow analyzer and/or related flow-based methods, emphasizing the minimum but adequate information that should be included in scientific or technical reports.

2. DESCRIPTION OF THE FLOW SYSTEM

Flow-based analytical techniques fall into one of four categories: air-segmented, unsegmented continuous flow, flow injection analysis, and sequential injection analysis. In air-segmented [4] and some earlier unsegmented [5–7] flow systems, samples are aspirated into a liquid moving stream at a fixed flow rate for a specific period of time. An operational feature of a segmented analyzer [4,8,9] is that air bubbles are used to segment the sample and reagent mixtures to facilitate mixing and minimize dispersion.

Flow injection analysis [8–11] involves the rapid injection of a sample into a continuously flowing unsegmented carrier stream. One or more reagent solutions are continuously merged with the carrier prior to detection. The injected sample zone undergoes dispersion and is mixed with the carrier and reagent solutions. The resultant product is transported through a flow-through detector for measurement and then to waste. The dispersion or dilution of the sample zone can be controlled and adapted to the required analysis, by the optimization of several factors including the injected sample volume, the flow rate of carrier and reagent streams, the reaction coil length, and the inner diameter of the tubing.

In sequential injection systems [8,9,12–14], a stack of well-defined unsegmented zones is assembled in a holding coil using a pump or liquid driver and a selection device. On transporting this stack of zones to the detector, the zones penetrate one another and mixing between their components gives rise to one or more detectable species. These products are measured as the zone stack passes through a suitable flow-through detector. The multiposition selection valve allows sequential injection analytical systems to be extremely versatile with many changes to the methodology being achievable through software control of the system parameters rather than actual physical changes to the hardware. Each port of the valve can be dedicated to a specific purpose, and the combinations of sample, standards, reagents, and detectors around the valve are easily modified to suit a particular analysis. The basic components

of the system are a liquid driver with only one carrier stream, a multiposition selection valve and a detector.

Multicommutation and binary sampling can be implemented in both unsegmented or segmented flow systems, and potentialities and limitations have been pointed out [15].

Detailed basic schematic diagrams of the different main approaches are very well illustrated [4–7,9,11], and are not repeated here.

For a complete description of a flow system, the following elements should be considered and described.

2.1 Flow pattern (technique)

Regarding flow techniques, the earlier flow analyzers based on Skeggs' concept [4] exploited air-segmented streams. Segmentation was at the time believed to be essential for minimizing sample carryover, improving mixing conditions, and scrubbing the tubing inner walls, and was attained by merging the flowing sample stream with an air stream. Further proposals exploiting segmentation by different gases or solvents have also been presented. The feasibility of using unsegmented flow became evident in the 1970s [7,10,16–18]: flow systems without segmentation presented enhanced versatility [19] and the term "flow injection analysis" was coined by Ruzička and Hansen [10]. Besides the flow injection analyzers, other flow systems based on unsegmented streams have been proposed [12,13,20–24] and the versatility is in general a consequence of the unsegmented streams. Mono-segmentation was further conceived [25] as an alternative to attain long sample residence times associated with low sample dispersion.

In this context, van der Linden presented a logical and descriptive classification scheme to match the various analytical systems [1]. For instance, "flow injection analysis could be described as an 'unsegmented flow system with injection of (test) sample' or 'unsegmented flow system with injection of reagents', and continuous flow analysis denoted as a 'segmented flow system with aspiration of (test) sample'. A flow injection system based on merging zones of analyte and reagent solution was indicated as an 'unsegmented flow system with injection of (test) sample and reagent'. A process monitor with a constant speed of (test) sample and based on the continuous flow analysis principle could be indicated as 'segmented flow system with continuous sampling'. This approach to classification and nomenclature has the additional advantage of being easily expanded. Thus, the nature of the segmenting medium can be easily added (air/nitrogen/argon…segmented flow system...) also liquid/liquid extraction can be conveniently incorporated, e.g., (toluene) segmented flow extraction system with sample injection". Examples for air [26] and organic solvent [27] carriers are given in detail in the literature. The absence of information about the flow technique implies unsegmented streams, and when the segmentor is not specified, segmentation by air is implied.

2.2 Stream parameters

Several stream parameters are critical in defining a particular flow-based analytical procedure. These include flow rate, volumes, and composition. If the stream propulsion mechanism is relevant, then it should be described.

Stream flow rates should be always specified, as well as temporal flow rate variations inherent to specific systems including the sample stopping [28], sinusoidal flows [29], intermittent streams [30], flow reversal [9,12,13,31], gradient flows [32], or combined flow rates (e.g., fast before sample arrival to the detector and slow during measurement). Indication of less-critical flow rates, such as the sample aspiration, which is usually an irrelevant parameter, is not recommended.

Sample volumes and the size of different reagent zones clearly have a direct impact on the performance of a flow-based procedure. A clear description of relevant zones is required. The size of

reagent zones is often dependent on the composition and concentration of the reagent. Therefore, in addition to volume, reagent composition and concentration must also be specified.

Composition of the flowing streams is usually strictly dependent on the constitution and flow rates of the pumped solutions, and on the sample handling, this latter being strongly related to the dispersions involved. Hence, it is important to specify clearly the preparation of the sample, carrier, and/or reagent solutions

It is important to describe how fluids are propelled, and the main liquid drivers such as a peristaltic pump [4], piston pump [12], gas propeller [33], or other possibilities for this task, such as those involving electro-osmotic flow [34], or gravitational flow [7], should be clearly stated. The peristaltic pump is by far the most used instrument for fluid propelling, therefore, absence of information is taken to imply use of this pump. Characteristics of pump tubing are mentioned in most publications, but this information is not needed unless there exist incompatibility between propelled fluids and pumping tubes.

2.3 Sample introduction (with possibility of reagent introduction)

The type of sample introduction should always be clearly specified [9]. In air-segmented [4] and some unsegmented [5–7] flow systems, samples are aspirated at a fixed flow rate for a specific period into the flow system. In flow injection systems [10,11], finite volumes of samples are injected into an unsegmented carrier stream. In flow injection systems, the injected sample volume should always be given, regardless of whether loop-based or time-based injection [35] is concerned. The dimensions of the injecting element (ex: length of an external loop), injection time, and other specific injection characteristics (simultaneous [36,37], sequential [38,39], sample splitting [36], multiple [40], tandem [41–44], hydrodynamic [45], nested [46]) should be also outlined. In sequential injection systems [9,12–14], samples are aspirated into an unsegmented stream for a specific period at a fixed flow rate using a selection device. Care should be taken to distinguish between the sequential injection determination of samples in a flow injection system [38,39] and an actual sequential injection analysis system [9,12–14] to avoid confusion. If possible, the sequential injection determination together with flow injection should be specified. Absence of information in this regard is taken to imply actual sequential injection analysis. Whenever necessary, the nature of the injection device and any auto-sampler should be described. Sample volume is one of the critical variables considered when designing flow injection or sequential injection systems, and a simple convenient method [47] is available for measuring the injection volume in flow injection and the aspirating volume in sequential injection systems.

2.4 Manifold

Tubing for building up the manifold should always be specified, but less emphasis should be given to manifold portions outside the analytical path. There are different types of reactors [1], and they should be specified. The coiled reactor is by far the most commonly used, and no further information means that this kind of reactor has been used. Tube length and tubing inner diameter as well as the winding diameter of the coiled reactor should always be specified.

Tubing material should be specified where it affects wetability by analyzer fluids to an extent where it impacts on the analysis.

Accessories such as mixing chambers, resin mini-columns, immobilized enzymes, connectors, etc., in the analytical path should be fully described only when used for the first time, otherwise citation is enough.

The flow diagram should be fully and clearly described in a diagram outlining, for example, the complete manifold, relative positions of components, relative tubing dimensions, flow rates, sample volumes, etc. Although the use of icons for representing some components should strongly be discouraged, in cases where they are used, the icons should clearly be described in the legend or text.

A literature survey reveals that numerous acronyms and/or abbreviations have been proposed for characterizing flow analyzers with specific, often minor, modifications in the manifold. In this regard, the use of IUPAC nomenclature should strongly be recommended. Otherwise, this policy must be strongly discouraged.

2.5 Sample processing

It is very important to emphasize the processes to which the sample is subjected, and the mean sample residence time with the corresponding flow rate should be recorded. In special situations, for example, when a catalytic method is concerned, the residence time of the sample being processed in specific portions of the manifold should be given.

It is advisable to report sample dispersion, especially in relation to flow injection systems, although contribution to dispersion by manifold components such as the valve and detector flow cell may contribute the dispersion number. For this purpose, the dispersion coefficient [11] has been used. The volumetric fraction [48] is a practical index to roughly determine the composition of any fluid element of the system in any part of the manifold any time after sample introduction.

Additional information is the completion degree of the physicochemical processes involved. This aspect has often been omitted perhaps because most of the flow analyzers operate in a transient manner and attainment of chemical equilibrium is often not necessary. For some applications, such as those based on relatively slow chemical reactions, statement on the degree of completeness is recommended.

In sequential injection systems the fundamental requirement to succeed is to achieve maximum zone penetration through a deliberate increase in axial dispersion, obtained by means of the flow reversal and channel design [12,14,49–52]. It is therefore very important that a device sequence [13,14,53] for one cycle of a sequential injection system is always given, including the exact time development of the method with proper description of the functions of the liquid driver and selection device. The device sequence can be conveniently given in the form of a table with a selection of the following columns that are applicable; time, valve port number or stream, pump or liquid driver operation (forward/reverse/stop) or alternatively flow direction (aspirate/dispense/stop), detector, short description, volume, flow rate, etc.

2.6 Detection

The internal volume of the detector flow cell should be specified with a description of its geometry. In most of the applications, detector dead volume (volume equivalent to attain ca. 67 % of the steady-state signal after a step-wise concentration variation [54]) and/or response time (related to the detector ability of fully monitoring a transient concentration variation [55]) are not critical for characterizing a flow system. These parameters should be specified only if they play a relevant role in recorded peak shape. It is advisable to include other parameters related to the detector such as wavelength, electrode size, sweep rate, excitation wavelength, etc.

Signal processing [3,11,56] is a very important part in flow and sequential injection systems, and the shape and quality of the analytical signal obtained may have a major influence on the performance of a flow or sequential injection analyzer. Regarding interpretation of the analytical signal, it is important to specify how the analytical signal is achieved (e.g., steady state, peak height, peak area, or peak width at a defined height). An important characteristic that should always be specified is the signal processing/recording/treatment. Detailed description of software for data acquisition (including signal filtering), feedback mechanisms, and/or calculation algorithms are recommended if they are original, otherwise proper citation is preferred.

3. PERFORMANCE OF THE FLOW SYSTEM

Besides the above-mentioned essential procedural aspects, it is important also to provide the main figures of merit for proper description of a flow-based procedure.

3.1 Sampling rate

The number of (test-) samples processed per unit time should be estimated in association with a specified carry-over level. Absence of information means that carry-over is not measurable. It is recommended to specify the consumption of sample and/or reagent when discussing sampling frequency, especially when less available or potentially environmentally hazardous reagents are concerned.

3.2 Analytical characteristics

Accuracy, sensitivity, detection limit, selectivity, dynamic range, and precision should be calculated from the data according to the IUPAC recommendation [3]. The system should be clearly validated using a standard method, with confidence limits and tests of significance [3,57]. There are several approaches to reporting detection limit. It is therefore important to clearly specify which formula is used [3].

Sample matrices play an important role in the performance of any flow-based analyzer. It is therefore important to clearly specify the maximum tolerance of all interferences involved in any dedicated flow-based analytical procedure.

3.3 Robustness

System robustness [58] can be discussed under two points of view. Firstly, the dependence of the analytical signal on variations of system parameters, that is, how the procedure is affected by slight variations in certain parameters such as flow rates, temperature, reagent concentrations, etc. The second relates robustness with instrumental stability, manifesting it-self as shift and/or drift in baseline and/or analytical signal. Stability of the analytical curve with time, cost, and maintenance requirements should be provided.

3.4 Portability

Another feature that could be usefully discussed in reporting about a flow system is its portability to other operational environments and consequent suitability for, e.g., in situ, in vivo, or on-site [2] analyses, as process analyzer or for on-line monitoring [2].

4. FINAL REMARKS

After the flow-based system has been characterized, it is recommended to provide some additional information. This should mainly be that related to some specific characteristics or potentialities such as, for example, zone sampling [59], merging zones and how to get them [35], sample electrolytic dissolution [60], in-line gas sampling [61], multi-commutation [43], etc., as well as its adherence to well-established variants of flow analysis, such as continuous flow, flow injection, sequential injection [1], and others.

The above-mentioned aspects may constitute a memorandum/list. Once it is taken into account for describing a novel flow-based methodology, one expects a tendency toward the development of systems that are more consistently designed. In addition, the goal to have a protocol for flow analysis will certainly be approached.

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